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Determination of Microquantity of Ethylphenylephrine in Plasma by Radioisotope Derivative Method using *p*-Toluenesulfonyl[^{35}S] Chloride[1)]

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A determination of microquantity of ethylphenylephrine in plasma of dogs and human volunteers after oral administration of a therapeutic dose was investigated by the radioisotope derivative method using *p*-toluenesulfonyl[^{35}S] chloride (tosyl[^{35}S] chloride). Tosyl chloride, in a weak alkaline medium containing 33% acetone, reacted with the phenolic hydroxyl and amino group of ethylphenylephrine molecule, producing N-tosylethylphenylephrine tosylate, and reacted with the phenolic hydroxyl group of the drug in a neutral medium, producing ethylphenylephrine tosylate. A linear correlation was found between the amount of ethylphenylephrine (0.0125—0.1 μg) added to human plasma and the radioactivity of N-tosylethylphenylephrine tosylate-^{35}S derivative produced from ethylphenylephrine which was extracted from the plasma samples.

After the administration of ethylphenylephrine in dogs, about 56—80% of total plasma level was found as unchanged drug in plasma. On the contrary, unchanged ethylphenylephrine was not detected in human plasma after oral administration of the drug. After oral administration of the sustained-release capsule, plasma level was compared with that of the uncoated tablet in man and it was found that increase of plasma level was slower and more durable after administration of the sustained-release capsule than that of the uncoated tablet. Thus, the radioisotope derivative method using tosyl[^{35}S] chloride was useful for determination of a microquantity of ethylphenylephrine or its metabolite in plasma.

The fluorometric determination of plasma concentration of ethylphenylephrine in man after oral administration of its therapeutic dose of two dosage forms was impossible for the reason of low drug level.[3)] The radioisotope derivative method has been applied for the determination of microquantity of biological materials in tissue[4)] and drugs or their metabolites in blood.[5)] Therefore, the present work was planned for the determination of a microquantity of ethylphenylephrine in plasma of dogs and man after the administration of a therapeutic dose of ethylphenylephrine preparations, by using *p*-toluenesulfonyl[^{35}S] chloride (tosyl[^{35}S] chloride).

Material and Method

Material——Tosyl[^{35}S] chloride (Radiochemical Centre, Amersham, England) was used. The specific radioactivity was 29—108 mCi/mmole for different preparations and the isotopic purity was ascertained

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2) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*

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by thin-layer chromatography (TLC) to be over 95% for all preparations. Ethylphenylephrine hydrochloride and two different dosage forms of ethylphenylephrine, the uncoated tablet and the sustained-release capsule, which were the same preparations as used in the previous work,[3] were used. Other reagents were all reagent grade and organic solvents were used after redistillation.

Silica gel F_{254} precoated (E. Merck A. G.) and three solvent systems (a) benzene–$CHCl_3$–AcOEt (8: 8: 3), (b) $CHCl_3$–AcOEt–ligroine (4: 4: 1), and (c) $CHCl_3$–benzene (1: 1) were used for TLC. Liquid scintillator was prepared from 3 g of 2,5-diphenyloxazole (PPO) and 0.2 g of 2,2′-*p*-phenylene-bis-(4-methyl-5-phenyloxazole) (dimethyl-POPOP) dissolved in 1 liter of toluene. Liquid scintillation spectrometer (Beckman Model LS-250), TLC-scanner (Aloka Radio-Chromatoscanner), spectrophotometer (Beckman Model DB), and ultraviolet ray detector (Nikko Seiki Works, Model LS-D3) were employed for the measurement or detection.

Tosylation with Non-labeled Tosyl Chloride——i) Effect of Alkalinity and Acetone Concentration on Tosylation: To 0.05 ml of aqueous ethylphenylephrine solution (1 mg/ml), 0.45 ml of 0.1M phosphate buffer (pH 7.0), 1% $NaHCO_3$, or 1% Na_2CO_3 was added and 0.1 ml of $(CH_3)_2CO$ solution of tosyl chloride (1 mg/ml) was mixed. Four kinds of alkaline mixture with $(CH_3)_2CO$ concentration of 10, 30, 50, and 75% in the final volume of 2 ml were made by addition of the required volume of $(CH_3)_2CO$ and H_2O. After standing for 2 hr at room temperature, the mixture was extracted three times with 1 ml each of hexane. The separated hexane layer was combined and evaporated to dryness on a steam bath. The residue was dissolved in a few drops of $CHCl_3$, spotted on a TLC plate, and developed with the solvent system (a). The adsorbent on chromatogram corresponding to *Rf* value of 0.1 or 0.3, detected under ultraviolet (UV) ray, was quantitatively transferred into a test tube and extracted twice with 2 ml each of AcOEt. The absorbance of the combined extract was measured at 274 nm.

ii) Effect of Concentration of $NaHCO_3$ on Tosylation: A mixture of 0.5 ml of aqueous solution of ethylphenylephrine (100 μg/ml) and 0.5 ml of 0.2, 1, or 5% $NaHCO_3$, added with 0.5 ml of $(CH_3)_2CO$ solution of tosyl chloride (200 μg/ml), was allowed to stand for 2 hr at room temperature and treated in the same way as the procedure described above.

iii) Effect of Volume of Reaction Mixture on Tosylation: The following mixtures were allowed to stand for 2 hr at room temperature and then treated in the same way as the procedure described above: (a) 0.5 ml of aqueous solution of ethylphenylephrine (100 μg/ml), 0.5 ml of 1% $NaHCO_3$, and 0.5 ml of $(CH_3)_2CO$ solution of tosyl chloride (200 μg/ml), (b) 1 ml of aqueous solution of ethylphenylephrine (50 μg/ml), 1 ml of 1% $NaHCO_3$, and 1 ml of $(CH_3)_2CO$ solution of tosyl chloride (100 μg/ml), (c) 2 ml of aqueous solution of ethylphenylephrine (25 μg/ml), 2 ml of 1% $NaHCO_3$, and 2 ml of $(CH_3)_2CO$ solution of tosyl chloride (50 μg/ml).

iv) Effect of Reaction Time on Tosylation: A mixture of 0.5 ml of ethylphenylephrine solution (100 μg/ml), 0.5 ml of 1% $NaHCO_3$, and 0.5 ml of tosyl chloride solution (200 μg/ml) was treated in the same way as above procedure after standing for 15, 30 or 120 min, or 18 hr at room temperature.

v) Isolation of Tosyl Derivatives: a) Aqueous solution of ethylphenylephrine (110 mg in 8 ml) was mixed with $(CH_3)_2CO$ solution of tosyl chloride (200 mg in 8 ml), and 8 ml of 1% $NaHCO_3$ was added. After shaking for 30 min, the mixture was extracted three times with 10 ml each of AcOEt. The combined extract was evaporated under a reduced pressure. The residue was dissolved in 0.5 ml of $CHCl_3$, spotted on a TLC plate for preparative thin-layer chromatography (PLC-plates Silica-gel F_{254} precoated, E. Merck A. G.), and developed with the solvent system (a). The adsorbent on the chromatogram corresponding to *Rf* value of 0.3, detected under UV ray, was extracted three times with 10 ml each of AcOEt. The residue after evaporation of the combined extract was dissolved in 0.5 ml of $CHCl_3$ and purified by rechromatography. The adsorbent zone of *Rf* value of 0.3 was extracted three times with 10 ml each of AcOEt. Evaporation of the combined AcOEt layer gave 122 mg of jelly-like residue. UV $\lambda_{shoulder}^{AcOEt}$ nm ($E_{1cm}^{1\%}$): 274 (16.4). NMR[6] (10% solution in $CDCl_3$) δ: 1.0 (3H, triplet), 2.4 (6H, singlet), 3.0—3.4 (4H, multiplet), 4.7—5.0 (1H, triplet), 6.8—7.6 (4H, multiplet), 7.3—7.9 (8H, quartet). Ferric chloride test,[7] negative. Test with sodium nitroprusside and acetaldehyde,[8] negative.

b) A mixture consisting of aqueous solution of ethylphenylephrine (110 mg in 8 ml), $(CH_3)_2CO$ solution of tosyl chloride (200 mg in 8 ml), and 8 ml of 0.5M phosphate buffer (pH 7.0) was treated in the same way as the procedure (a), except that the fraction of *Rf* value of 0.1 instead of 0.3 was extracted with AcOEt. Evaporation of the extract left 64 mg of jelly-like residue. UV λ_{max}^{AcOEt} nm ($E_{1cm}^{1\%}$): 274 (44.3). NMR (10% solution in $CDCl_3$) δ: 1.0 (3H, triplet), 2.4 (3H, singlet), 3.0—3.4 (4H, multiplet), 4.8 (2H, triplet and broad resonance), 6.8—7.6 (4H, multiplet), 7.4—8.0 (4H, quartet). Ferric chloride test, negative. Test with sodium nitroprusside and acetaldehyde, positive.

6) Nuclear magnetic resonance (NMR) spectra were obtained by the use of tetramethylsilane as an internal standard by Varian T-60 NMR spectrometer.
7) S. Soloway and S.H. Wilen, *Anal. Chem.*, **24**, 979 (1952).
8) F. Feigl, "Spot Tests," Vol. II, English Ed., Elsevier Publishing Co., New York, 1954, p. 189.

Tosylation with Tosyl[^{35}S] Chloride——i) Effect of Specific Radioactivity in the Reaction Mixture on Tosylation: To a mixture of 0.25 ml of aqueous solution of ethylphenylephrine (0.4 μg/ml) and 0.25 ml of 1% $NaHCO_3$, 0.25 ml of $(CH_3)_2CO$ solution of tosyl[^{35}S] chloride (12.5, 50, 100, or 200 μCi/ml) was added. Each mixture was allowed to stand at room temperature for 2 hr and extracted three times with 0.5 ml each of hexane containing 10 μg of a carrier[9] per ml. The combined hexane layer was evaporated to dryness on a steam bath with air current. The residue was dissolved in a few drops of $CHCl_3$, spotted on a TLC plate, and the plate was developed with the solvent system (a). The adsorbent on the chromatogram corresponding to the carrier, detected under UV ray, was quantitatively scraped off and transferred into a counting vial, and the radioactivity was counted after shaking in 10 ml of liquid scintillator. A mixture of 0.25 ml of H_2O, 0.25 ml of 1% $NaHCO_3$, and 0.25 ml of each concentration of $(CH_3)_2CO$ solution of tosyl[^{35}S] chloride was treated in the same way as the procedure described above for determination of the reagent blank.

ii) TLC of Tosyl Derivative obtained by Reaction with Tosyl[^{35}S] Chloride: A mixture of 0.25 ml of aqueous solution of ethylphenylephrine (0.4 μg/ml), 0.25 ml of 1% $NaHCO_3$, and 0.25 ml of $(CH_3)_2CO$ solution of tosyl[^{35}S] chloride (100 μCi/ml) was allowed to stand at room temperature for 2 hr. The reaction mixture was extracted three times with 0.5 ml each of hexane containing the carrier. The combined extract was evaporated on a water bath of 50° with air current. The residue, dissolved in a few drops of $CHCl_3$, was spotted on a TLC plate and the plate was developed with the solvent systems (a), (b), or (c). The position of the carrier on each chromatogram was detected under UV ray. The radioactivity on the plate was scanned by the TLC-scanner.

Determination of Ethylphenylephrine in Plasma——i) Free Ethylphenylephrine: A mixture of 1 ml of plasma sample, 1 ml of 1% $NaHCO_3$, and 1 ml of H_2O was shaken twice with 10 ml each of AcOEt. The combined AcOEt layer was evaporated to dryness under a reduced pressure. To the residue 0.5 ml of H_2O and 0.5 ml of $(CH_3)_2CO$ were added and the precipitate produced was removed by centrifugation. The supernatant, weakly acidified with 0.5 ml of 0.002N HCl, was shaken twice with 1 ml each of $(C_2H_5)_2O$ and hexane. Organic solvent layer was removed and aqueous $(CH_3)_2CO$ solution was evaporated to dryness under a reduced pressure. To the residue dissolved in 0.25 ml of H_2O, 0.25 ml of 1% $NaHCO_3$ and 0.25 ml of $(CH_3)_2CO$ solution of tosyl[^{35}S] chloride (100 μCi/ml) were added. The mixture was allowed to stand for 2 hr at room temperature and extracted three times with 0.5 ml each of hexane containing the carrier. The combined extract was evaporated on a water bath of 50° with air current. The residue was dissolved in a few drops of $CHCl_3$, spotted on a TLC plate, and the plate was developed with the solvent system (a). The adsorbent corresponding to the carrier, which was detected under UV ray, was quantitatively transferred to a counting vial containing 10 ml of liquid scintillator and the radioactivity was counted. Normal plasma, added with a required amount of ethylphenylephrine, was treated in the same way as above and a standard curve was preparaed.

ii) Total Ethylphenylephrine: A mixture of 1 ml of plasma sample, 1 ml of 0.5M phosphate buffer (pH 5.3), 0.5 ml of β-glucronidase solution (1600 units/ml of H_2O, type II, Nutritional Biochemicals Cooperation (N.B.C.)), and 2 drops of $CHCl_3$ was incubated at 37° for 18 hr. After adjusting to pH 8.0 by addition of 1N NaOH, the mixture was shaken twice with 10 ml each of AcOEt. The combined AcOEt layer was treated in the same way as the procedure for free ethylphenylephrine. The recovery of ethylphenylephrine added to normal plasma was $47.3 \pm 2.4\%$ ($n=3$).

Experiment in Dogs and Man——i) In Dogs: Three healthy beagle dogs weighing 10.2—11.1 kg were used after fasting about 18 hr. They were orally administered the uncoated tabelt in a dose equivalent to 5 mg of ethylphenylephrine. The blood sample was drawn from the front paw vein 1 and 4 hr after the administration, and the plasma was used for the assay of free and total ethylphenylephrine.

ii) In Man: Two healthy volunteers weighing 58 and 64 kg were used after fasting about 12 hr. They were administered orally the uncoated tablet in a dose equivalent to 15 mg of ethylphenylephrine. The blood was drawn from the forearm vein 1, 2, 4, 6, and 8 hr after ingestion of the drug for the assay of free and total ethylphenylephrine in the plasma. After 1 week, the sustained-release capsule was administered orally, and free and total ethylphenylephrine in the plasma samples was assayed.

Result

Tosylation using Non-labeled Tosyl Chloride

Table I shows the effect of alkalinity and acetone concentration in the reaction mixture on tosylation of ethylphenylephrine. The most intense absorbance at 274 nm was observed under the condition of addition of $NaHCO_3$ and 30—50% acetone concentration for the spot with *Rf* value of 0.3, indicating the highest formation of bitosylate. The formation of mono-

9) Tosyl derivative isolated in method v—a using non-labeled tosyl chloride.

Table I. Effect of Alkalinity and Acetone Concentration in Reaction Mixture on Tosylation of Ethylphenylephrine using Non-labeled Tosyl Chloride

Alkaline solution added	Amount of tosylate	Final acetone concentration (%, v/v) 10	30	50	75
0.1M phosphate buffer (pH 7.0)	bitosylate[a]	—[b]	0.009[c]	0.008	N.D.[d]
	monotosylate[e]	—	0.013	0.055	0.036
1% $NaHCO_3$	bitosylate	0.049	0.060	0.062	0.045
	monotosylate	N.D.	N.D.	N.D.	N.D.
1% Na_2CO_3	bitosylate	—	0.020	0.030	0.044
	monotosylate	—	N.D.	N.D.	N.D.

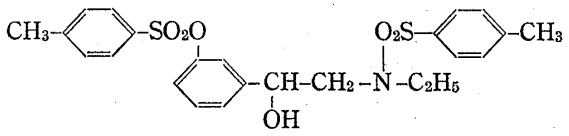
a) obtained from spot of *Rf* 0.3 b) —=not measured c) absorbance at 274 nm
d) N.D.=not detected e) obtained from spot of *Rf* 0.1

Table II. Effect of Concentration of $NaHCO_3$, Final Volume of Reaction Mixture, and Reaction Time on Formation of Ethylphenylephrine Bitosylate using Non-labeled Tosyl Chloride

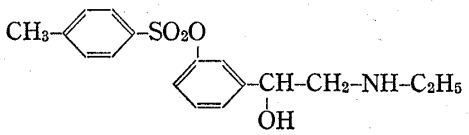
Concn. of $NaHCO_3$ added (%, w/v)	0.2	1	5	
Amount of bitosylate (Absorbance at 274 nm)	0.034	0.062	0.052	
Final Vol. of mixt. (ml)	1.5	3	6	
Amount of bitosylate (Absorbance at 274 nm)	0.057	0.052	0.037	
Reaction time (min)	15	30	120	18(hr)
Amount of bitosylate (Absorbance at 274 nm)	0.057	0.057	0.057	0.056

tosylate was detected only when the reaction was carried out with the addition of 0.1M phosphate buffer (pH 7.0). Moreover, it was evident from the result in Table II that the yield of bitosylate was the optimal under the reaction condition of the addition of 1% $NaHCO_3$ and a smaller volume (1.5 ml), but independent of the reaction time (15 min—18 hr).

Two tosyl derivatives isolated by using TLC for preparative thin-layer chromatography were identified as N-tosylethylphenylephrine tosylate (bitosylate) and ethylphenylephrine tosylate (monotosylate) from their NMR spectra and color reactions (Fig. 1).



(I) N-tosylethylphenylephrine tosylate (bitosylate)



(II) ethylphenylephrine tosylate (monotosylate)

Fig. 1. Tosyl Derivatives Isolated by TLC after Reaction of Ethylphenylephrine with Tosyl Chloride

(I) obtained from spot of *Rf* 0.3 by solvent system (a)
(II) obtained from spot of *Rf* 0.1 by solvent system (a)

From these results, the reaction condition of the addition of 1% $NaHCO_3$, final volume of 0.75 ml, final acetone concentration of 33%, and reaction time of 2 hr were used for tosylation of ethylphenylephrine with tosyl[^{35}S] chloride in subsequent experiments. For the detection of the spot of active bitosylate on TLC, N-tosylethylphenylephrine tosylate (bitosylate) was used as a carrier.

Table III. Effect of Specific Radioactivity in Reaction Mixture on Formation of Ethylphenylephrine Bitosylate using Tosyl [^{35}S] Chloride

Specific radioactivity (μCi/ml)	0.1 μg of drug (cpm)	Reagent blank (cpm)
3.125/0.75	5326	751
12.5 /0.75	27632	2678
25.0 /0.75	50477	5208
50.0 /0.75	93528	7102

Tosylation using Tosyl[^{35}S] Chloride

A higher specific radioactivity in the reaction mixture increased the radioactivity of the spot corresponding to the carrier, detected under UV ray, in the case of either ethylphenylephrine or reagent blank (Table III). The ratio of reagent blank (cpm) to 0.1 μg of ethylphenylephrine (cpm) was 0.141, 0.097, 0.103, and 0.076 for 3.125, 12.5, 25, and 50 of specific radioactivity (μCi/0.75 ml), respectively. The increase of the specific radioactivity tended to enhance the quantitative accuracy, but median specific radioactivity in this investigation, 25 μCi/0.75 ml, was used in subsequent experiments in order to avoid too high a value of the reagent blank.

The position of radioactivity of ethylphenylephrine tosyl-^{35}S derivative on TLC plates, detected by scanning TLC-scanner, after development with three solvent systems, agreed with the spot of the carrier detected under UV ray; *Rf* 0.30 for solvent system (a), 0.41 for solvent system (b), and 0.02 for solvent system (c).

Determination of Various Amounts of Ethylphenylephrine added to Plasma

A linear relationship was observed between the radioactivity subtracted with the blank value and the amount of ethylphenylephrine added to the dog plasma (0.025—0.1 μg/ml of plasma) and to the human plasma (0.0125—0.1 μg/ml of plasma) (Fig. 2). In the case of human plasma, the relationship of radioactivity and amount of ethylphenylephrine was

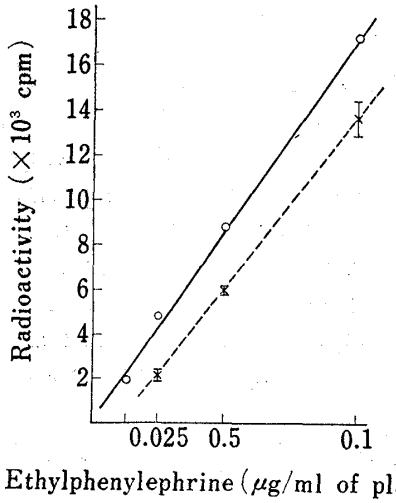


Fig. 2. Radioactivities in Dog and Human Plasma added Various Amounts of Ethylphenylephrine

—○—: human plasma
---×---: dog plasma, mean value of two experiments
Vertical lines indicate the range.

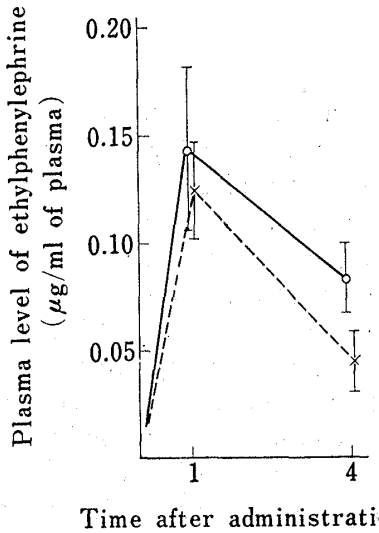


Fig. 3. Mean Plasma Levels of Total and Free Ethylphenylphrine after Oral Administration of Uncoated Tablet in Dogs

—○—: total ethylphenylephrine, $n=3$
---×---: free ethylphenylephrine, $n=3$
Vertical lines indicate the standard error of the mean.

obtained as the line through zero point, but the line tended to miss the zero point in the case of dog plasma. The radioactivity of dog plasma added with 0.025, 0.05, or 0.1 μg of ethylphenylephrine was reproducible in two repeated determinations.

Plasma Level of Ethylphenylephrine after Oral Administration of Ethylphenylephrine Dosage Forms in Dogs and Man

Fig. 3 shows plasma levels of total and free ethylphenylephrine after oral administration of an uncoated tablet equivalent to 5 mg of ethylphenylephrine in dogs. One hour after administration of the drug, mean plasma levels of 0.15 μg/ml in terms of total and 0.125 μg/ml in terms of free ethylphenylephrine were observed. Thus, about 80% of total ethylphenylephrine in dog plasma was detected as free ethylphenylephrine. About 56% of mean plasma level of total ethylphenylephrine (0.08 μg/ml) was detected as free ethylphenylephrine 4 hr after administration of the drug in dogs.

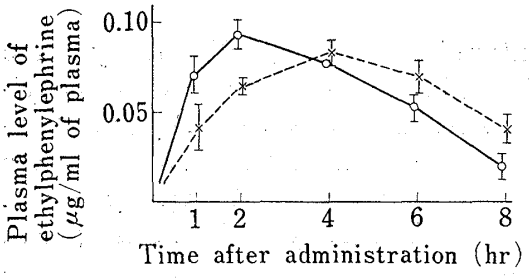


Fig. 4. Mean Plasma Levels of Total Ethylphenylephrine after Oral Administration of Uncoated Tablet and Sustained-release Capsule in Man

—○—: uncoated tablet, $n=2$
---×---: sustained-release capsule, $n=2$
Vertical lines indicate the range.

Free ethylphenylephrine in human plasma was not more than 0.01 μg/ml during the first 8 hr after administration of either the uncoated tablet or the sustained-release capsule. Thus, free ethylphenylephrine was practically undetectable in human plasma after the administration of ethylphenylephrine in a dose used therapeutically. The time courses of total ethylphenylephrine in human plasma after administration of the uncoated tablet and the sustained-release capsule are shown in Fig. 4. The mean level of ethylphenylephrine after oral administration of the uncoated tablet reached a peak level in 2 hr and declined thereafter. The peak plasma level was observed 4 hr after administration of the sustained-release capsule with the level rising gradually during 1 to 4 hr. This result indicated a rapid absorption and elimination of the uncoated tablet, and a gradual absorption of the sustained-release capsule.

Discussion

The determination of ethylphenylephrine in concentration less than 0.1 μg/ml of plasma was impossible by fluorometry.[3] In the present investigation, the radioisotope derivative method with tosyl[^{35}S] chloride was used for the determination of about 0.01 μg of ethylphenylephrine, and plasma level in man after oral administration of ethylphenylephrine dosage forms in a therapeutic dose was measurable. The time necessary for the peak plasma level of total ethylphenylephrine after oral administration of the uncoated tablet and the sustained-release capsule in man was 2 and 4 hr, respectively, in the present study. This result agrees with the fact that the urinary excretion rate of ethylphenylephrine was fastest during the first 0—2 hr after administration of the uncoated tablet and during 4—6 hr after administration of the sustained-release capsule in man.[3]

An interesting observation was that about 80 or 56% of total ethylphenylephrine was detected as free ethylphenylephrine in dog plasma, but free ethylphenylephrine was not detected in human plasma after administration of the drug. In the previous study,[3] about 60% of the amount excreted was detected as free ethylphenylephrine in dog urine but free ethylphenylephrine was not detected in human urine. These results suggest that the metabolic mechanism of ethylphenylephrine might be different between a dog and man. More detailed study will be necessary in the future to clarify this problem.

Acetic[^{3}H] anhydride, which reacts with amino group of a compound, has been used as a reagent for radioisotope derivative method for the determination of microquantity of a drug or its metabolite in blood.[5b-e] Aizawa, *et al.*[4g] used tosyl[^{35}S] chloride for the determination of noradrenaline in rat brain by the radioisotope derivative method, but they did not clarify its reaction mechanism. The present work showed that tosyl chloride chiefly reacts with the phenolic hydroxyl group of ethylphenylephrine in the reaction medium added with phosphate buffer solution (pH 7.0), and reacts with the phenolic hydroxyl group and amino group of this compound in an alkaline medium added with 1% $NaHCO_3$ or 1% Na_2CO_3. Acetone concentration and volume of the reaction mixture also affected tosylation of ethylphenylephrine.

Kunzman, *et al.*[5e] stated, in their experiment on radioisotope derivative method using acetic[^{3}H] anhydride, that a standard curve should be prepared for every experiment, since the radioactivity obtained from plasma added with 0.025 to 1.0 μg of pseudoephedrine was not linear. In the present study, the standard curve obtained from dog plasma added with less than 0.025 to 0.1 μg of ethylphenylephrine also showed a tendency of nonlinearity, though the standard curve obtained from human plasma showed a linearity through zero point. The standard curve, however, was prepared for every experiment, since the specific radioactivity of tosyl[^{35}S] chloride in the reaction mixture affected tosylation of ethylphenylephrine.

In the present study, some of the substances extracted from plasma by ethyl acetate also reacted with tosyl[^{35}S] chloride and interfered the isolation of ethylphenylephrine tosyl derivative on TLC. For that reason, purification of the extracts from plasma, such as elimination of precipitate produced dy addition of acetone and extraction by ether and hexane, was necessary in the stage of extraction of ethylphenylephrine from plasma samples. Thus, 0.0125 μg of ethylphenylephrine was capable of being separated from plasma components.

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